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Ether Phosphatidylcholines: Comparison of Miscibility with Ester Phosphatidylcholines and Sphingomyelin, Vesicle Fusion, and Association with Apolipoprotein A-I†

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Received June 19, 1986; Revised Manuscript Received August 14, 1986

ABSTRACT: Nonhydrolyzable matrices of ether-linked phosphatidylcholines (PCs) and sphingomyelin have been used to study the mechanism of action of lipolytic enzymes. Since ether PCs, sphingomyelin, and ester PCs vary in the number of hydrogen bond donors and acceptors in the carbonyl region of the bilayer, we have examined several physical properties of ether PCs and sphingomyelin in model systems to validate their suitability as nonhydrolyzable lipid matrices. The intermolecular interactions of ether PCs with ester PCs, sphingomyelin, and cholesterol were investigated by differential scanning calorimetry. Phase diagrams constructed from the temperature dependence of the gel to liquid-crystalline phase transition of 1,2-*O*-dihexadecyl-*sn*-glycero-3-phosphocholine (DPPC-ether) and 1,2-*O*-ditetradecyl-*sn*-glycero-3-phosphocholine (DMPC-ether) with both 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) demonstrated complete lipid miscibility in the gel and liquid-crystalline phases. Additionally, phase diagrams of egg yolk sphingomyelin (EYSM) with DMPC or DMPC-ether and 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) or 1,2-*O*-dioctadecyl-*sn*-glycero-3-phosphocholine (DSPC-ether) demonstrated no major differences in miscibility of EYSM in ester and ether PCs. The effect of 10 mol % cholesterol on the thermal transitions of mixtures of ester and ether PCs also indicates little preference of cholesterol for either lipid. The fusion of small single bilayer vesicles of DMPC, DMPC-ether, DPPC, and DPPC-ether to larger aggregates as determined by gel filtration indicated that the ester PC vesicles were somewhat more stable. The rate of association of apolipoprotein A-I with DMPC or DMPC-ether multilamellar liposomes was compared. The rate was fastest at the gel → liquid-crystalline transition temperature (T_c) of either lipid and was consistent with the insertion of the protein into lattice defects in the lipid matrix. Ether PCs interact with ester PCs, sphingomyelin, cholesterol, and apolipoproteins in a manner similar to ester PCs. The interaction between these lipids appears to be dominated by hydrocarbon chain interactions instead of the hydrogen bonding groups in the carbonyl region. Thus, ether PCs appear to be suitable analogues of the ester PCs for the elucidation of structural–functional mechanisms of lipolysis.

Lipolytic enzymes play an essential role in the metabolism of biological membranes and plasma lipoproteins. Kinetic studies on lipolytic enzymes are difficult to interpret because (a) the processes occur in a heterogeneous medium at a lipid–water interface, (b) the composition and physical properties of the interface are continuously altered by the hydrolysis of the substrate, and (c) the accumulation of lipolytic products can produce subdomains that may have different physical properties (Scanu et al., 1982; Brockman, 1984). To maintain a constant physical state of the lipid–water interface in model substrate systems, nonhydrolyzable lipid matrices of sphin-

† This research was supported by grants from the National Institutes of Health (HL 27341, HL 30913, HL 30914, and T32-HL 07582).

0006-2960/86/0425-7711$01.50/0

gomyelin or ether phosphatidylcholines (PCs)[1] have been used (Pownall et al., 1985a). The nonhydrolyzable analogues of ester PCs, which are competitive inhibitors of lipolytic enzymes, allow the separation of equilibrium binding and catalytic steps (Massey et al., 1985a; Pownall et al., 1985b; Burns et al., 1981; McClean & Jackson, 1985).

To alleviate problems in interpretation of kinetic experiments on lipolytic enzyme model systems, the physical properties of the ether and ester phospholipids must be similar, such that segregation of ether PCs or sphingomyelin into lipid subdomains or the formation of packing defects that enhance lipolytic enzyme activity or protein association is minimal (Pownall et al., 1981). Unlike ester PCs, ether PCs lack the carbonyl groups at the site of bonding to the glycerol backbone. As a consequence, there is decreased hydrogen bonding between ether PCs that permits tighter packing of the hydrocarbon moieties and gives a slight increase in transition enthalpy and in transition temperature (Eibl, 1984). Sphingomyelin differs from the ether and ester PCs in that it contains both a hydrogen bond donor and acceptor in the carbonyl or "hydrogen belt region" (Brockerhoff, 1982). In the present report, we show by several physicochemical criteria, which include properties of the gel to liquid-crystalline phase transition, vesicle fusion, and the kinetics of protein–lipid association, that the ether and ester lipids have almost identical behavior.

Experimental Procedures

Materials

Apolipoprotein A-I and DMPC-ether were prepared as previously described (Pownall et al., 1978; Massey et al., 1985a). DPPC-ether and DSPC-ether were obtained from Bachem (Bubendorf, Switzerland). DMPC, DPPC, DSPC, and EYSM were obtained from Avanti Polar Lipids (Birmingham, AL). Tritiated phosphatidylcholines were labeled by demethylation and remethylation with [^{3}H]CH_3I (Patel et al., 1979). All phospholipids were examined for purity by thin-layer chromatography prior to use with $CHCl_3$/MeOH/H_2O (65:35:4 v/v) as the solvent system. [^{14}C]-Glucose was from New England Nuclear (Boston, MA).

Methods

Differential Scanning Calorimetry. To prepare the samples at various molar ratios, solutions of the lipids in CH_3Cl were mixed, dried under a stream of nitrogen, dissolved in benzene, frozen at −70 °C, and lyophilized overnight. A standard buffer consisting of 10 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, and 1 mM NaN_3, pH 7.4, was added to suspend the dry samples. Each sample was measured at a scanning rate of 30 °C/h in a MicroCal MC-2 differential scanning calorimeter (Amherst, MA). Phase diagrams were constructed from the half-height peak width of the main phase transition as function of the molar percentage of the lower melting phospholipid. The enthalpy of the transition was determined by integration of the area under the main transition. Ideal phase diagrams were calculated according to Mabrey and Sturtevant (1976).

[1] Abbreviations: PC, phosphatidylcholine; EYSM, egg yolk sphingomyelin; DSPC-ether, 1,2-*O*-dioctadecyl-*sn*-glycero-3-phosphocholine; DPPC-ether, 1,2-*O*-dihexadecyl-*sn*-glycero-3-phosphocholine; DMPC-ether, 1,2-*O*-ditetradecyl-*sn*-glycero-3-phosphocholine; DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; apoA-I, apolipoprotein A-I, the most abundant protein of human plasma high-density lipoproteins; T_c, temperature of the midpoint of the gel → liquid-crystalline transition; ΔH, enthalpy of the gel → liquid-crystalline transition; DSC, differential scanning calorimetry; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid; ESR, electron spin resonance.

Table I: Enthalpy and Transition Temperature Values of the Gel → Liquid-Crystalline Phase Transition As Measured by DSC

lipid	enthalpy (kcal/mol)	T_C (°C)
DMPC	6.3	23.1
DMPC-ether	7.5	26.2
DPPC	7.7	40.6
DPPC-ether	9.8	42.8
DSPC	9.8	53.7
DSPC-ether	12.9	55.8
EYSM	5.3	37.7

Gel Filtration Chromatography. The fusion of small unilamellar phospholipid vesicles to larger aggregates was followed by gel filtration chromatography (Wong et al., 1982; Wong & Thompson, 1981). Single bilayer vesicles (50 mM) were prepared by sonication for 45 min using a Branson Model 350 sonifier (Danbury, CT). The samples were centrifuged at 108000*g* to remove titanium fragments, and following centrifugation, the upper two-thirds of each tube was removed and analyzed to determine phospholipid concentration (Bartlett, 1959). The vesicles were analyzed on a 1.6 × 30 cm water-jacketed Sepharose CL-2B (Pharmacia, Upsala, Sweden) column where the temperature was maintained by a circulating water bath. Elution profiles of the phospholipid samples were determined by liquid scintillation counting. The column was calibrated by using multilamellar vesicles to determine the void volume and [^{14}C]glucose to determine the included volume. To initiate the experiment samples, single bilayer vesicles free of multilamellar vesicles were diluted with warm standard buffer to a final concentration of 30 mM phospholipid, sealed under nitrogen in disposable microcentrifuge tubes, and stored at room temperature. At 10, 20, and 30 days, the samples were rechromatographed. Control samples were kept above T_c.

Kinetics of Apolipoprotein A-I Association. The rate of apoA-I association with multilamellar liposomes of DMPC or DMPC-ether was measured by the rate of decrease of liposomal turbidity (Pownall et al., 1978, 1981). For these measurements, the phospholipid (0.5 mg/mL) and apo A-I (0.25 mg/mL) were suspended in a buffer consisting of 8.5% KBr, 1 mM NaN_3, 1 mM EDTA, and 10 mM Tris-HCl, pH 7.4. Equal volumes of phospholipid and apoA-I solution were preincubated at the appropriate temperature and then mixed. Rates of association were determined by following the reduction in right angle light scattering vs. time, measured with an SLM 8000 spectrofluorometer (Urbana, IL) with an excitation and emission wavelength of 325 nm. The data were treated as a pseudo-first-order kinetic process, where we defined a rate constant $k_{1/2} = 1/\tau_{1/2}$, where $\tau_{1/2}$ represents the time for disappearance of 50% of the initial light-scattering intensity.

Results

Ether and Ester Phosphatidylcholine Interaction. The enthalpy and midpoint temperatures of the gel to liquid-crystalline phase transition for the phospholipids studied were measured by differential scanning calorimetry (Table I). The ether phospholipids have both higher enthalpies and higher T_c's as compared to their ester counterparts with the same hydrocarbon chain length. Both T_c and ΔH increased linearly as a function of diacyl and dialkyl chain length for the respective ester and ether PCs. Thermograms for mixtures of DMPC and DPPC (Figure 1A) gave a single peak for the main gel → liquid-crystal transition and an increase in T_c as the proportion of DPPC in the mixture increased. There was also a gradual increase in the width of the transitions as the amounts of DPPC and DMPC approached equimolar. Mix-

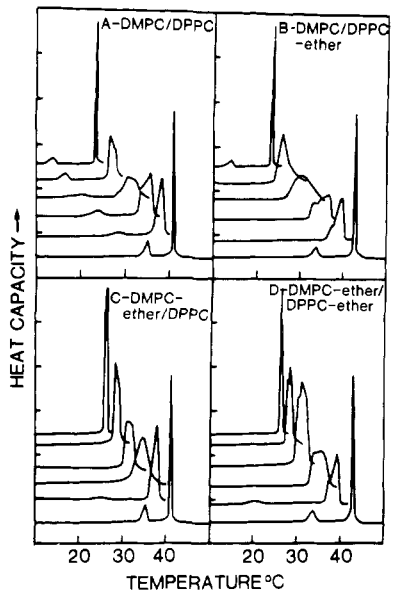

FIGURE 1: DSC thermograms of DMPC/DPPC (panel A), DMPC/DPPC-ether (panel B), DMPC-ether/DPPC (panel C), and DMPC-ether/DPPC-ether (panel D) mixtures. Phospholipid (10 mM) was suspended in a buffer of 10 mM Tris, pH 7.4, containing 100 mM NaCl, 1 mM EDTA, and 1 mM sodium azide. The samples were heated at a rate of 30 °C/h. Molar ratios of 100:0, 80:20, 60:40, 40:60, 20:80, and 0:100 were used.

tures of DMPC/DPPC-ether (Figure 1B), which have the greatest difference in T_c and ΔH (Table I), show a broad asymmetric main transition as the ratio of the lipids approached unity. DMPC-ether and DPPC mixtures (Figure 1C) have the smallest difference in T_c and ΔH and had the most symmetric main transition. Melting curves for DMPC-ether/DPPC-ether mixtures (Figure 1D) show the transition of the higher melting lipid, DPPC-ether, predominates over the lower melting DMPC-ether even when the majority of the lipid in the mixture is DMPC-ether. Both the enthalpy values (Figure 2A) and the T_c (Figure 2A, inset) values for DMPC/DPPC vs. DMPC-ether/DPPC and DMPC/DPPC-ether vs. DMPC-ether/DPPC-ether (Figure 2A, inset) displayed a linear increase as the proportion of the higher melting lipid in the mixture increased. A pretransition (Figure 1A) was observed for all of the DMPC/DPPC mixtures, which increased in temperature to parallel that of the main transition. For DMPC and DPPC-ether (Figure 1B) mixtures, a pretransition was observed only for the 100% DMPC and DPPC-ether samples. For DMPC-ether/DPPC mixtures (Figure 1C) and DMPC-ether/DPPC-ether (Figure 1D) mixtures, a pretransition appears only at high molar percentages of the higher melting lipid.

The phase diagrams for mixtures of DMPC/DPPC (Figure 3A), DMPC/DPPC-ether (Figure 3B), DMPC-ether/DPPC (Figure 3C), and DMPC-ether/DPPC-ether (Figure 3D) illustrate that all four lipid pairs were miscible in both the gel and liquid-crystalline phases. DPPC mixtures with DMPC and DMPC-ether displayed less deviation from ideal behavior than mixtures with DPPC-ether.

Phosphatidylcholine–Cholesterol Interaction. The interaction of cholesterol with mixtures of ether and ester PCs was measured by DSC. DSC traces (Figure 4) of 10 mol % cholesterol in 40:60 mol % mixtures of DMPC/DPPC, DMPC/DPPC-ether, DMPC-ether/DPPC, and DMPC-ether/DPPC-ether were similar in appearance with T_c values of 33.6–33.9 °C. Each thermogram displayed a broad shoulder on the higher melting side of a sharper transition.

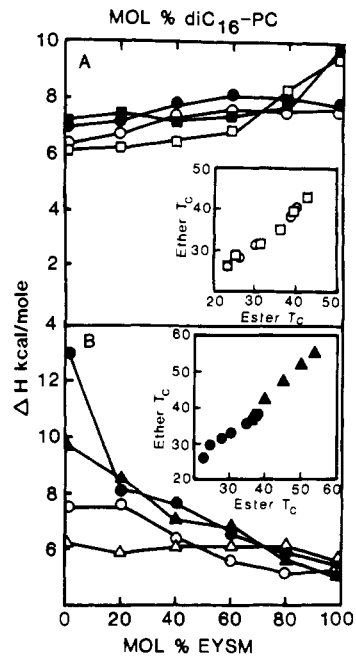

FIGURE 2: Enthalpy values of the main phase transition for mixtures of DMPC/DPPC (○), DMPC/DPPC-ether (□), DMPC-ether/DPPC (●), and DMPC-ether/DPPC-ether (■) obtained from the area under the thermograms in Figure 1 (panel A). The T_c values for DMPC-ether/DPPC vs. DMPC/DPPC (○) and for DMPC/DPPC-ether vs. DMPC-ether/DPPC-ether (□) from Figure 1 are plotted in the insert. Enthalpy values of the main transition for mixtures of DMPC/EYSM (△), DMPC-ether/EYSM (○), DSPC/EYSM (▲), and DSPC-ether/EYSM (●) obtained from the areas under the thermograms in Figures 5 and 6 (panel B). The T_c values for DMPC/EYSM vs. DMPC-ether/EYSM (●) and for DSPC/EYSM vs. DSPC-ether/EYSM (▲) from Figures 5 and 6 are plotted in the insert.

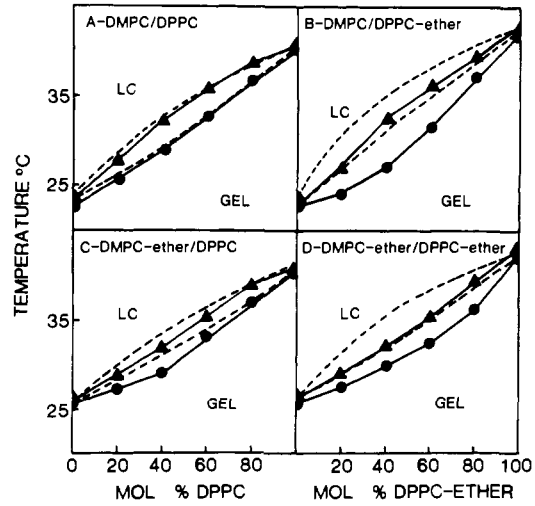

FIGURE 3: Phase diagrams constructed from the upper (▲) and lower (●) temperature limits of the main transition at half-height in Figure 1 for mixtures of DMPC/DPPC (panel A), DMPC/DPPC-ether (panel B), DMPC-ether/DPPC (panel C), and DMPC-ether/DPPC-ether (panel D). The dashed lines show the calculated theoretical solidus and liquidus lines obtained by assuming that mixing is ideal in both the gel and liquid-crystalline states.

Phosphatidylcholine–Sphingomyelin Interaction. Mixtures of DMPC and DMPC-ether with egg yolk sphingomyelin, where sphingomyelin was the higher melting lipid (Figure 5), demonstrated a single symmetric peak for the main thermal transition, and an increase in T_c with sphingomyelin content. The DMPC-ether/EYSM transition peaks were sharper and more symmetric than the DMPC/EYSM transitions presumably due to the smaller difference in T_c between DMPC-ether

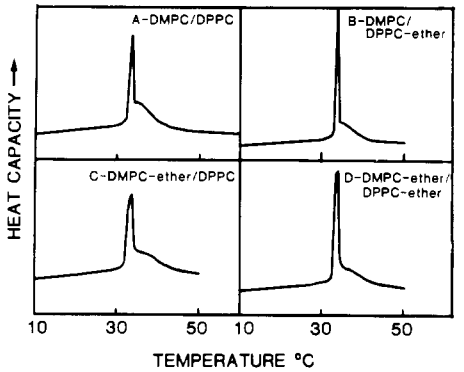

FIGURE 4: DSC thermograms for mixtures of 10 mol % cholesterol and mixtures of 10 mM phospholipid at a ratio of 60:40; DMPC/DPPC (panel A); DMPC/DPPC-ether (panel B); DMPC-ether/DPPC (panel C); DMPC-ether/DPPC-ether (panel D).

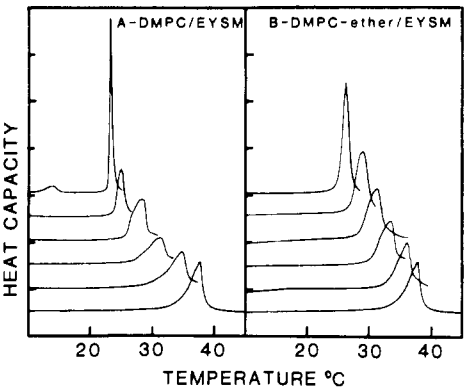

FIGURE 5: DSC thermograms for DMPC/EYSM (panel A) and DMPC-ether/EYSM (panel B) mixtures at molar ratios of 100:0, 80:20, 60:40, 40:60, 20:80, and 0:100. The lipid mixtures at a concentration of 4 mM were heated at 30 °C/h.

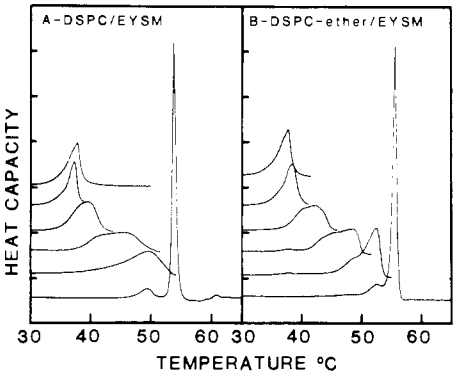

FIGURE 6: DSC thermograms for mixtures of DSPC/EYSM (panel A) and DSPC-ether/EYSM (panel B) run under conditions similar to Figure 3. The phospholipid concentration was 4 mM.

and EYSM. DSPC/EYSM and DSPC-ether/EYSM mixtures (Figure 6), where the PC was the higher melting lipid, also produced a single main transition peak; however, at high molar percentages of the PCs, the thermograms are much broader than seen with high molar percentages of DMPC and DMPC-ether due to the greater difference in ΔH and T_c between the higher melting lipid and EYSM. The phase diagrams for mixtures of ester and ether PCs with EYSM (Figure 7) demonstrate similar miscibility for the PC pairs. However, for DSPC and DSPC-ether, the degree of gel-state immiscibility with EYSM was more pronounced. For DSPC-ether, the pretransition appears as a shoulder of the main transition, whereas with DSPC a pretransition is evident only for the neat lipid. Enthalpies for DMPC, DMPC-ether, DSPC, and DSPC-ether with egg yolk sphingomyelin (Figure

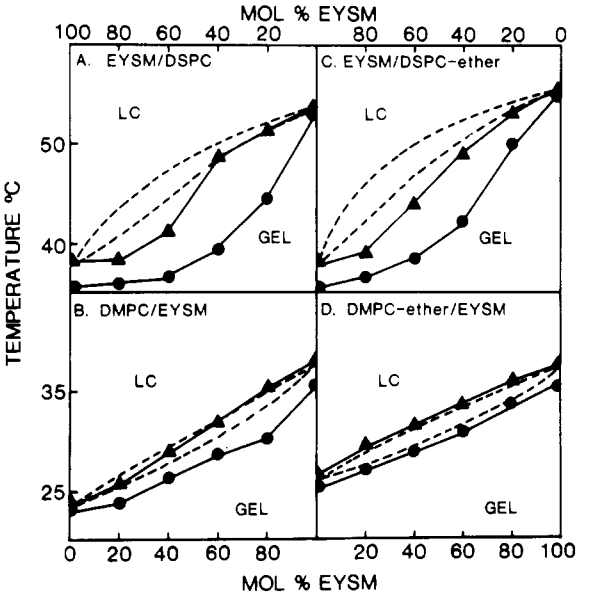

FIGURE 7: Phase diagrams constructed from the upper (▲) and lower (●) temperature limits of the main transition at half-height in Figures 5 and 6 for mixtures of DMPC/EYSM (panel A), DMPC-ether/EYSM (panel B), DSPC/EYSM (panel C), and DSPC-ether/EYSM (panel D). The dashed lines are the calculated ideal phase diagrams.

2B) were proportional to the enthalpic contribution of each lipid in the pair. A linear dependence of T_c with the proportion of the higher melting lipid (Figure 2B, inset) was evident for DMPC/EYSM vs. DMPC-ether/EYSM and for DSPC/EYSM vs. DSPC-ether/EYSM mixtures.

Stability of Single Bilayer Vesicles. The stability of sonicated single bilayer vesicles made from ester and ether PCs with time was investigated by gel filtration chromatography. Concentrated solutions (30 mM) of DMPC, DMPC-ether, DPPC, and DPPC-ether were incubated for 0, 10, 20, and 30 days at ambient temperature (≃24 °C) after which they were analyzed by chromatography on Sepharose CL-4B. The elution profiles at 0- and 30-days incubation are shown in Figure 8 for the four PCs. For DMPC (Figure 8A), the vesicles become more heterogeneous and increase in size as indicated by a broadening of the elution profile and earlier elution of the sample. The width of the elution profile for the higher melting ester, DPPC vesicles (Figure 8B), at 30 days is broader than that of DMPC and was shifted more toward the void volume. The elution profile for DMPC-ether vesicles (Figure 8C) at 30 days was much broader than its ester analogue with more of the lipid eluting in the void volume. Vesicles of DPPC-ether (Figure 8D) have the largest percentage of lipid eluting in the void volume after 30 days of storage at room temperature.

Kinetics of Apolipoprotein A-I Protein Association. The rate of apoA-I association with multilamellar vesicles of DMPC and DMPC-ether was followed by changes in turbidity caused by the formation of small optically clear lipid–protein recombinants as a function of time. The rate of reduction of light scattering was temperature dependent and varied by 3 orders of magnitude over a narrow temperature range. An Arrhenius plot (Figure 9) exhibited maxima at 24 °C for DMPC and 27.5 °C for DMPC-ether, the respective transition temperatures of these two lipids. Above and below these temperatures, the rate declined rapidly. A slightly higher maximal rate of association for DMPC-ether ($t_{1/2}$ = 0.25 min) was found as compared to DMPC ($t_{1/2}$ = 0.92 min). The rate of association of apoA-I with DPPC and DPPC-ether was very slow even at their transition temperatures.

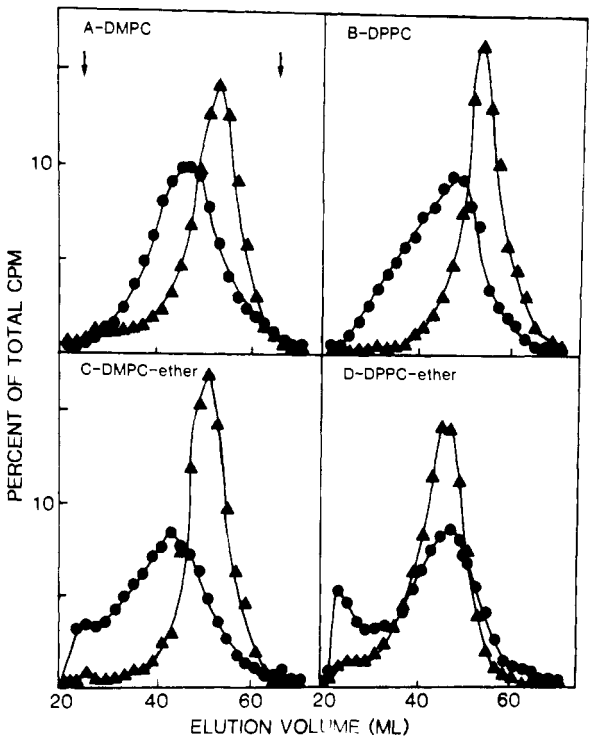

FIGURE 8: Elution profiles of single bilayer vesicles at 0 (▲) and 30 (●) days storage at room temperature. The vesicles (1 mL) of 30 mM phospholipid were chromatographed on a 1.6 × 30 cm CL-2B column and eluted with standard buffer. Fractions (1 mL) were collected and counted by liquid scintillation counting. Profiles were plotted as the percent of total counts vs. elution volume for DMPC (panel A), DMPC-ether (panel B), DPPC (panel C), and DPPC-ether (panel D). The arrow on the left (panel A) is the void volume of the column, while the arrow on the right is the included volume.

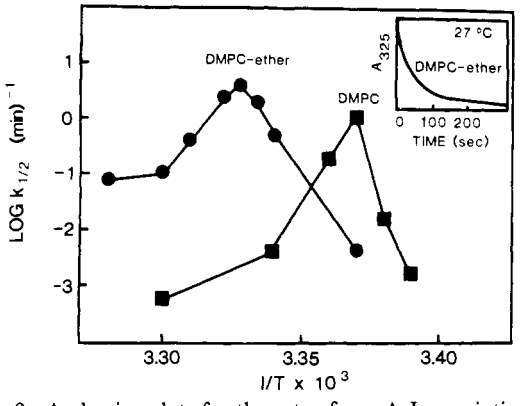

FIGURE 9: Arrhenius plots for the rate of apo A-I association with multilamellar suspensions of DMPC (■) and DMPC-ether (●). The rate constant $k_{1/2}$ represents the time for a 50% reduction in right angle light scattering at 325 nm. Apo A-I (0.25 mg/mL) dissolved in a 8.5% KBr, 1 mM NaN_3, 1 mM EDTA, and 1 mM Tris-HCl, pH 7.4, was mixed with an equal volume of phospholipid (0.5 mg/mL) in the same buffer. A typical kinetic trace for the reduction in right angle light scattering by apolipoprotein A-I association with DMPC-ether multilamellar liposomes at 27 °C is shown in the insert.

Discussion

For ether lipids to be a reliable tool in the investigation of lipolytic enzyme mechanisms, they must retain maximal structural and functional homology with their ester analogues. Studies on the lipid–lipid interaction of DMPC with DPPC, as measured by DSC, ESR, and fluorescence techniques (Mabrey & Sturtevant, 1976; Shimsick & McConnell, 1973; Parasassi et al., 1984), have demonstrated nearly ideal mixing of the two lipids. The thermograms for the mixtures of DMPC/DPPC, DMPC/DPPC-ether, DMPC-ether/DPPC, and DMPC-ether/DPPC-ether (Figure 1) were characterized by a single main transition peak. This result indicates that, while all of the lipids may not mix ideally they are essentially miscible. The enthalpy values and transition temperatures (Figure 2A) for the four lipid pairs were linearly dependent upon the proportional contribution of each lipid in the mixture, as expected for miscible lipid pairs. The phase diagrams in Figure 3 demonstrate graphically that the four lipid pairs are miscible in both the gel and liquid-crystalline states. Mixtures of DMPC and DMPC-ether with DPPC displayed little deviation from ideal mixing, while the mixtures with DPPC-ether had a greater degree of deviation from ideal behavior due to the greater enthalpic contribution of the ether.

If ether PCs are to be viable structural analogues of ester PCs, there must be no preferential partitioning of cholesterol into either lipid in a mixed lipid complex (van Dijck et al., 1976). The sharp thermal transition peak (Figure 4) represents a pure phospholipid phase, while the broad shoulder is due to the formation of a cholesterol–phospholipid complex of unknown structure and stoichiometry (Estep et al., 1978). In the system studied here, there is no preferential partitioning of cholesterol between ether and ester phospholipids because both phospholipids are miscible in the gel and liquid-crystalline phases.

Sphingomyelin, the second most abundant phospholipid in the surface monolayer of lipoproteins (Bradley & Gotto, 1978), exhibits both compositional and temperature-dependent lateral phase separation in sphingomyelin–phosphatidylcholine mixtures (Untracht & Shipley, 1977). EYSM (Figures 5 and 6) is miscible with both the lower melting DMPC and DMPC-ether and the higher melting DSPC and DSPC-ether over the entire range of composition. The EYSM mixtures with DMPC and its ether analogue (Figure 7) displayed less deviation from ideal behavior than DSPC and DSPC-ether/EYSM mixtures presumably due to smaller differences in enthalpies. Transition temperatures and enthalpies (Figure 2B) for both the ether–ester PC and the phosphatidylcholine–EYSM mixtures were linearly dependent upon the proportional contribution of each lipid in the mixture, as expected for miscible lipid pairs.

The stability of sonicated single bilayer ester and ether vesicles was investigated (Figure 8) by gel filtration chromatography. The greater extent of aggregation or fusion of the ether PC single bilayer vesicles may be due to a combination of the strain imposed upon the bilayer structure due to the high degree of curvature produced by sonication and the lack of a hydrogen bonding carbonyl group. DPPC and DPPC-ether exhibited a greater shift toward the void volume over DMPC and DMPC-ether probably due to their higher transition temperature (Larrabee, 1979). Overall, it appears that the ether lipid single bilayer vesicles tend to be somewhat less stable than those made from the ester.

The maximal rate of apolipoprotein–PC complex formation (Figure 9) occurs at the gel → liquid-crystalline transition temperature, presumably due to the occurrence of maximal numbers of lattice defects or vacancies in the lipid bilayer (Pownall & Massey, 1982; Pownall et al., 1978). The slightly higher overall rate of apoA-I binding to DMPC-ether suspensions may be attributable to a lesser degree of hydrogen bonding between the individual ether phospholipid molecules which allows easier insertion of the apoproteins into the lattice defects.

The physical properties of ether PCs have been examined in monolayer studies (Demel et al., 1984), by nuclear magnetic resonance (Bittman et al., 1981) and fluorescence probe

techniques (Massey et al., 1985b). The current studies provide additional proof that the similar physical properties of ether and ester PCs are a valuable tool for examining the mechanisms of lipid hydrolysis and lipolytic enzyme association with lipid particles.

ACKNOWLEDGMENTS

We thank Marjorie Sampel for preparation of the manuscript and Susan Kelly for providing the line drawings.

Registry No. DMPC, 18194-24-6; DMPC-ether, 36314-48-4; DPPC, 63-89-8; DPPC-ether, 36314-47-3; DSPC, 816-94-4; DSPC-ether, 1188-85-8; cholesterol, 57-88-5.

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